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Sparsentan Improves Glomerular Blood Flow and Augments Protective Tissue Remodeling in Mouse Models of Focal Segmental Glomerulosclerosis (FSGS)

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Background

- Preliminary nonclinical and emerging clinical evidence indicate strong antiproteinuric actions of sparsentan, a unique dual Endothelin Angiotensin Receptor Antagonist (DEARA).
- The effects of this highly selective antagonism of both endothelin A receptor (ET_AR) and the angiotensin II subtype 1 receptor (AT_1R) has been more pronounced in experimental and clinical settings compared to current standard of care using an AT_1R blocker (ARB).
- Considering the broad spectrum of renal actions of endothelin-1 (ET-1) and angiotensin II (Ang II), inhibition of both pathways using sparsentan is postulated to target multiple renal cell types by a variety of mechanisms to protect podocytes and preserve kidney function.

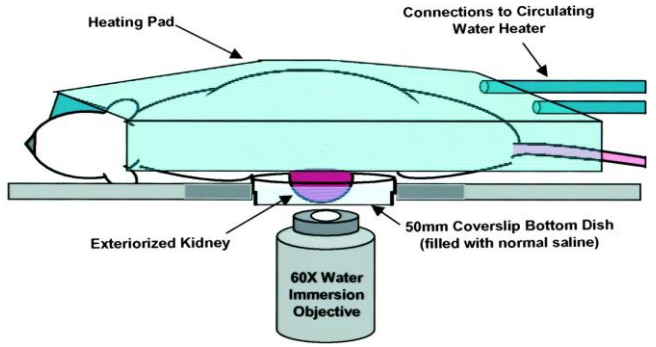
Aim

The aim of this nonclinical study was to determine glomerular mode of action of sparsentan as compared to the ARB losartan by direct visualization of effects on renal hemodynamics and tissue remodeling in the intact living mouse kidney.

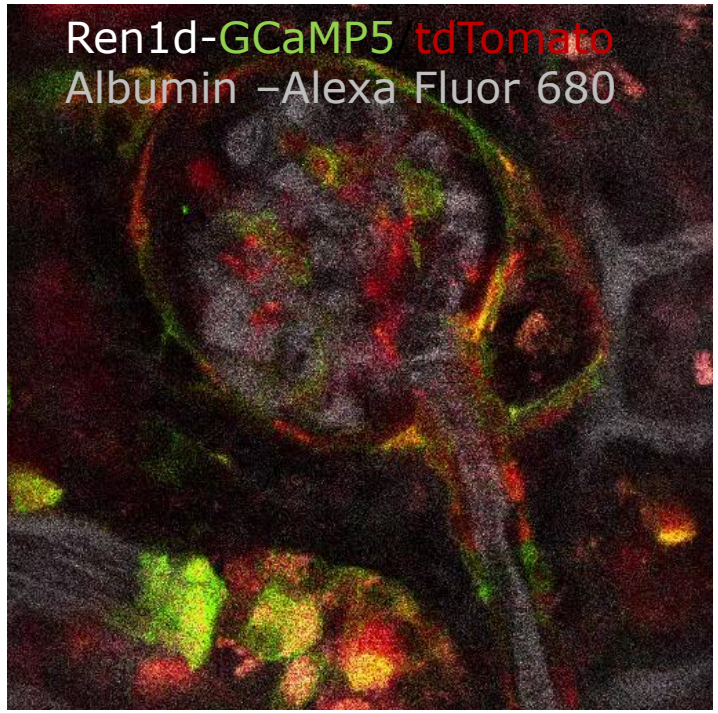
Methods

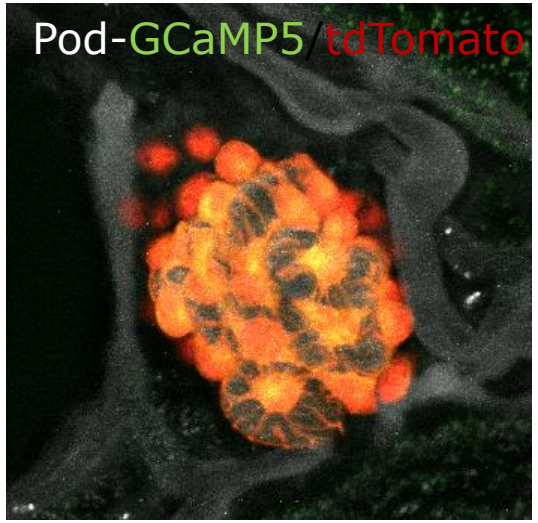
- Transgenic (Tg) Mouse Models:
  - Healthy-Tg:
    Ren1d-GCaMP5/tdTomato
  - FSGS-Tg:
    Pod-GCaMP5/tdTomato/
    TRPC6 TG (1.5 years)[1]
- Intravital multiphoton imaging:
  - Glomerular hemodynamics
  - Tissue remodeling
- Classic phenotyping:
  - Albuminuria
  - Histology

[1]Krall P et al *PLoS One*. 2010.



Dunn et al *AJP Cell Physiol*. 2002.
Kang et al *AJP Renal Physiol*. 2006.





Measured parameters:

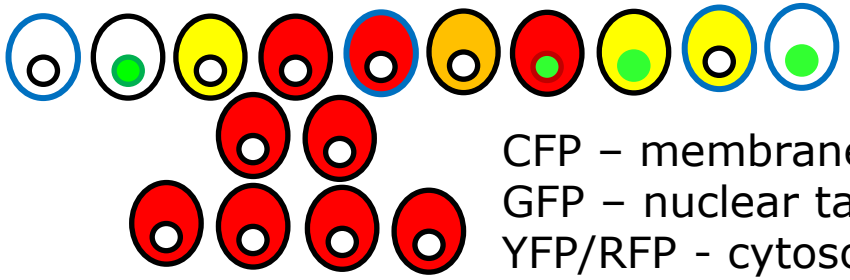
- Single Nephron GFR
- Blood flow
- Albumin glomerular sieving coefficient
- Afferent and Efferent/ glomerular diameter
- Vascular Smooth Muscle Cell/Podocyte calcium

Methods

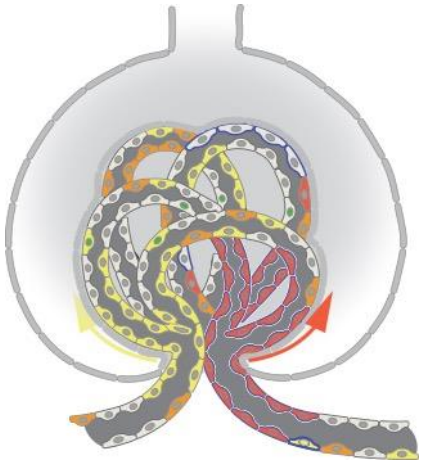
Transgenic (Tg) Mouse Models
to track tissue remodeling:

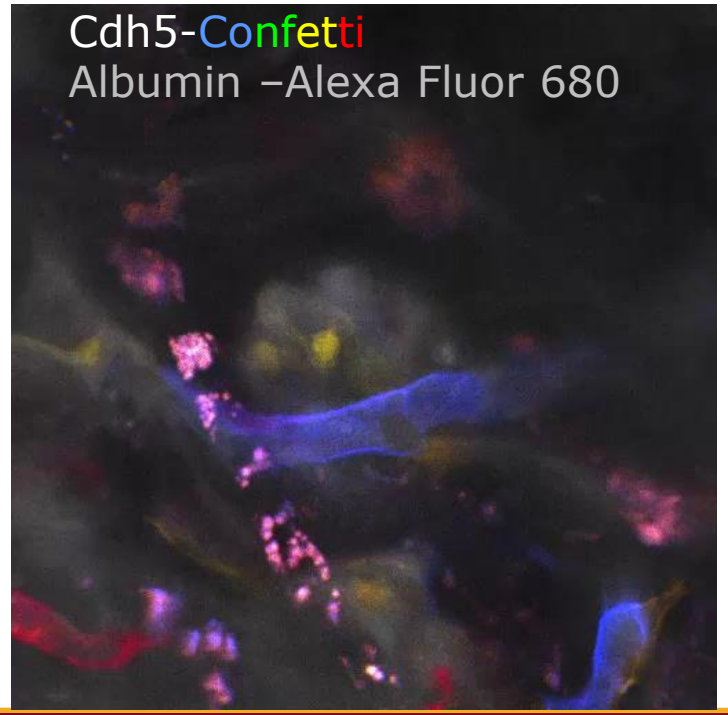
- Healthy Confetti Tg:
  - Ren1d-Confetti (mesenchymal cells)[2]
  - Cdh5-Confetti (endothelial cells)[3]

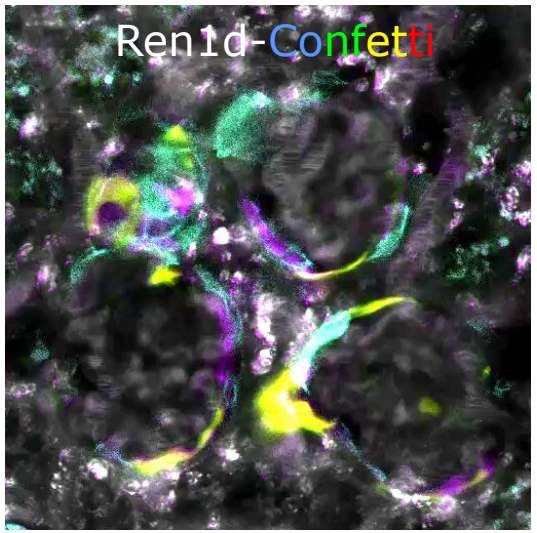
Multicolor CFP/GFP/YFP/RFP reporter
that allows single cell ID and fate tracking



CFP – membrane targeted
GFP – nuclear targeted
YFP/RFP - cytosolic







Measured parameters:

- Confetti cell number
- Clone number
- Confetti cell number/clone

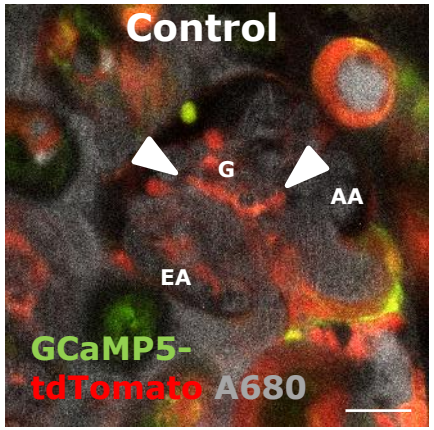
[2]Kaverina NV et al *PLoS One* 2017.
[3]Desposito D et al *JCI Insight* 2021.

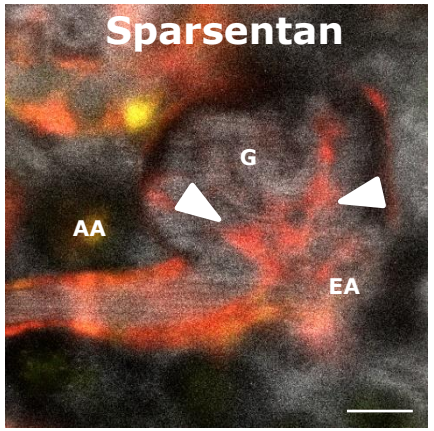
Results

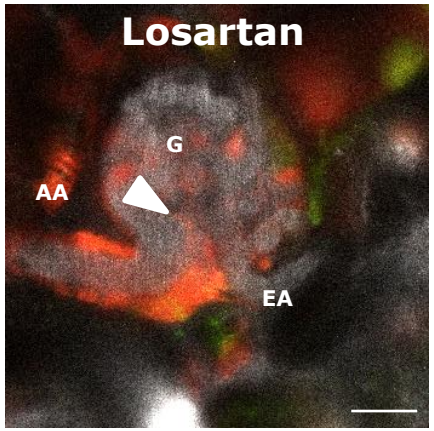
Treatment for 6 weeks po. **MPM imaging**

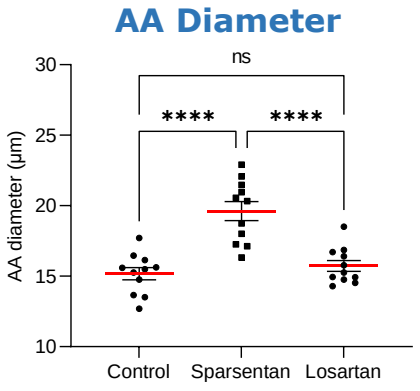
Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

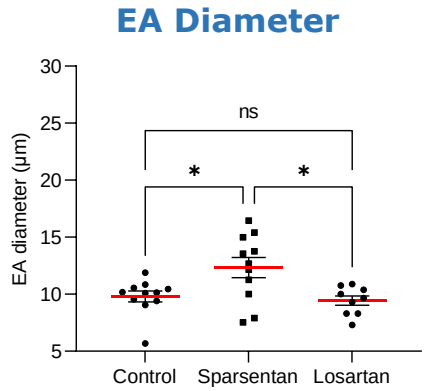
Healthy-Tg mice: basal renal hemodynamic responses

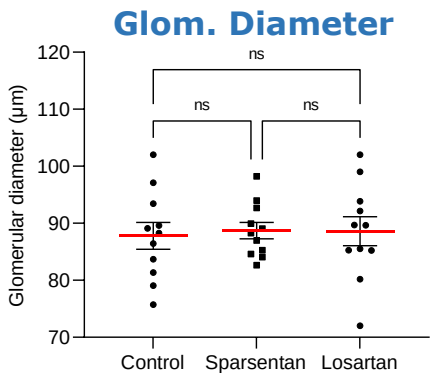


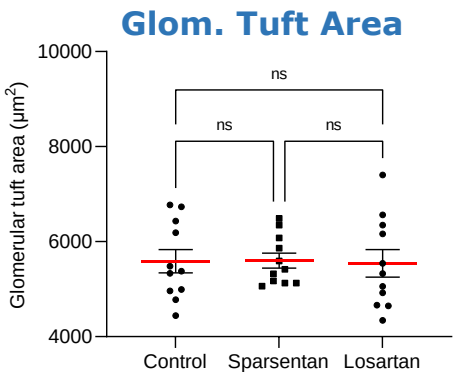


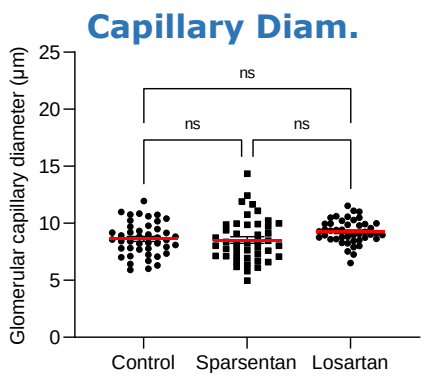


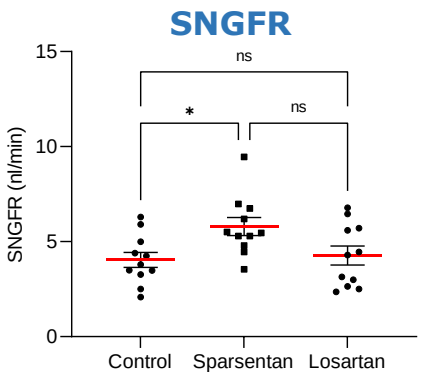


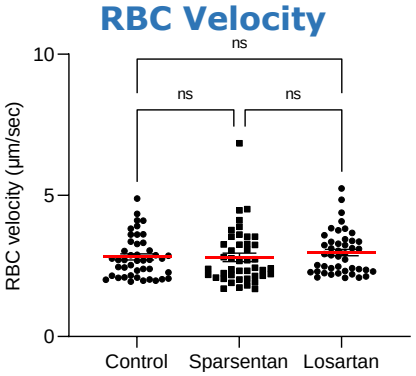












Sparsentan-treatment significantly ($p<0.05$) increased AA and EA diameters and SNGFR vs. control

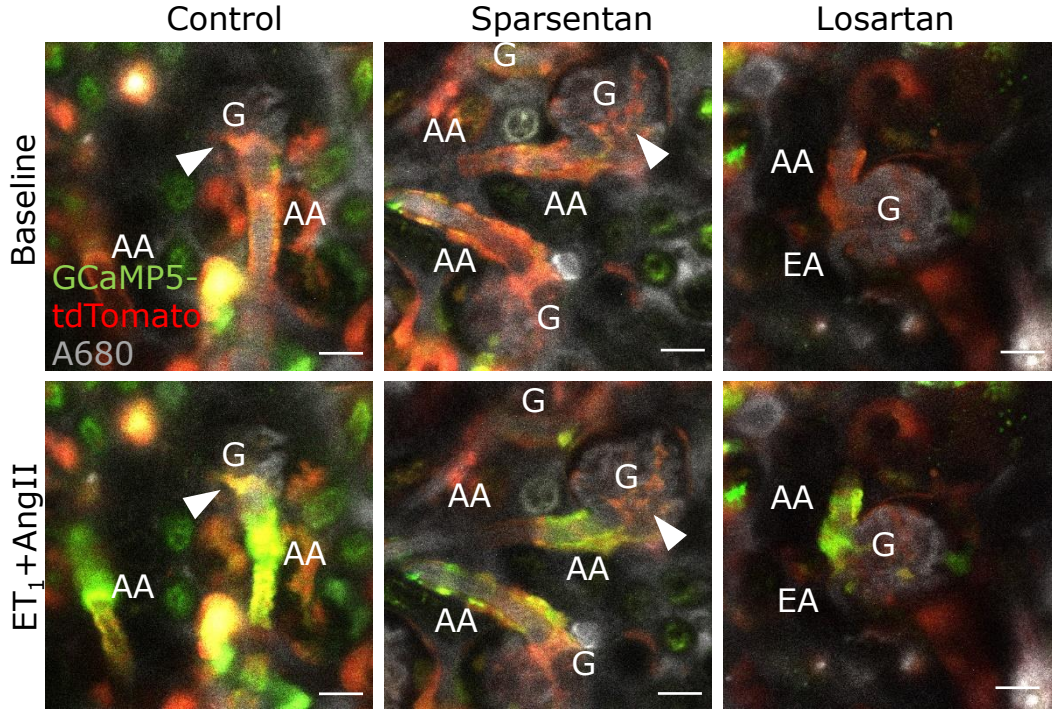
Results

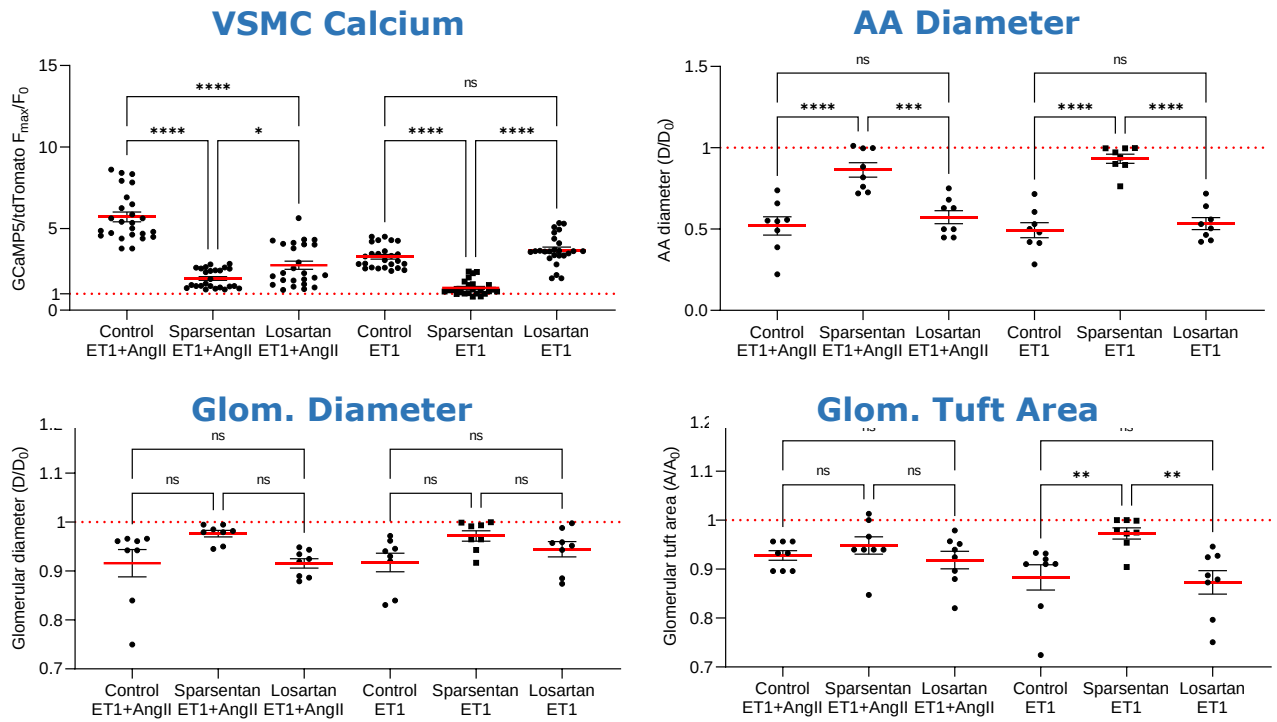
Treatment for 6 weeks po. **MPM imaging**

Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

Acute vaso-constriction: ET-1 w/wo Ang II ia.

Healthy-Tg mice: Hemodynamic responses to acute agonist-induced vasoconstriction

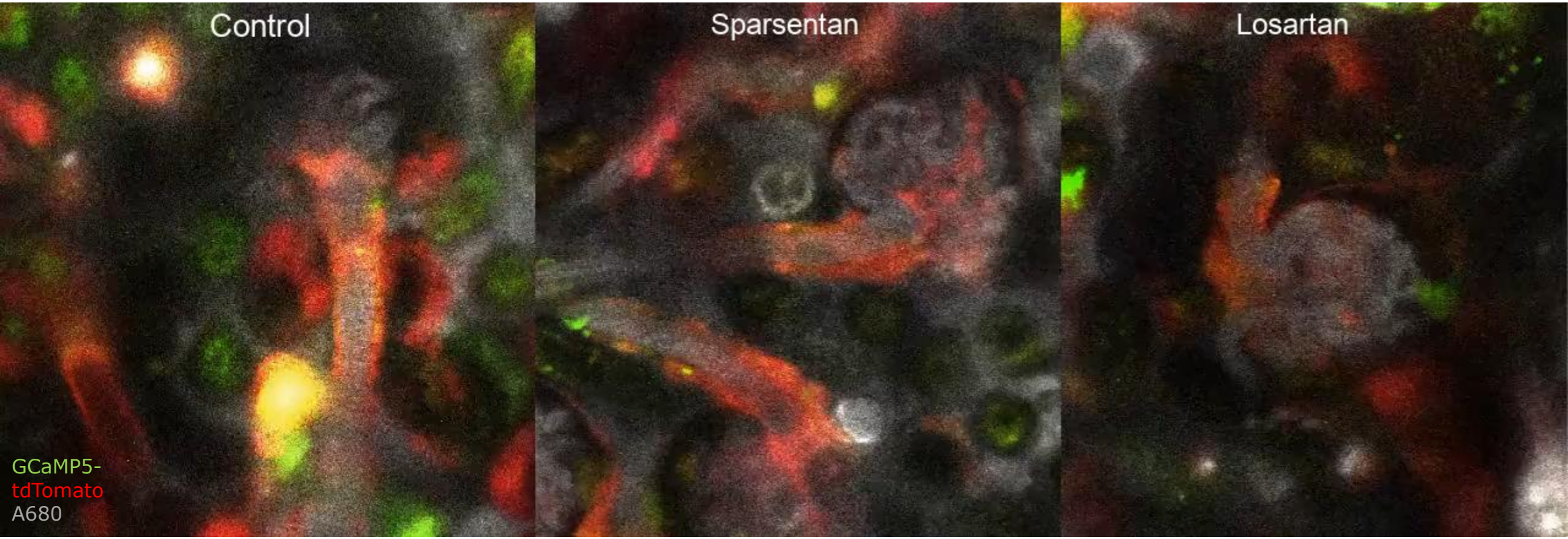




Sparsentan and Losartan inhibited acute ET + Ang II–induced VSMC Ca2+ elevations. Only sparsentan inhibited acute ET + Ang II–induced AA vasoconstriction. Losartan had no effect on any of the ET-1 only-induced glomerular hemodynamic alterations, in contrast to sparsentan.

Representative videos of agonist-induced hemodynamics

Acute vasoconstriction by ET-1+Ang II injection ia.



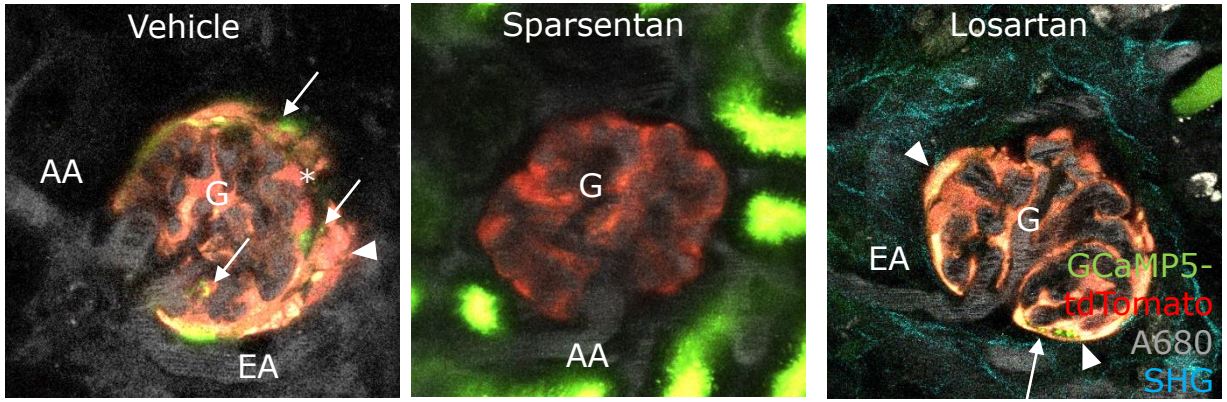
Results

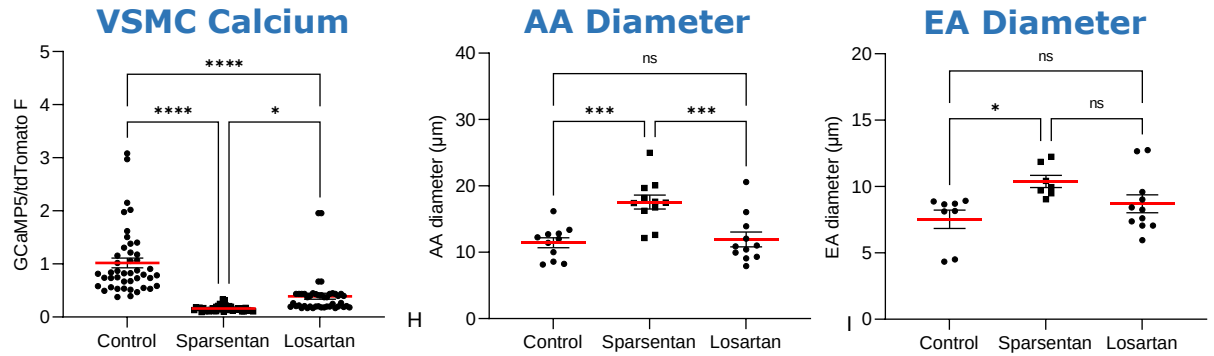
Treatment for 6 weeks po.

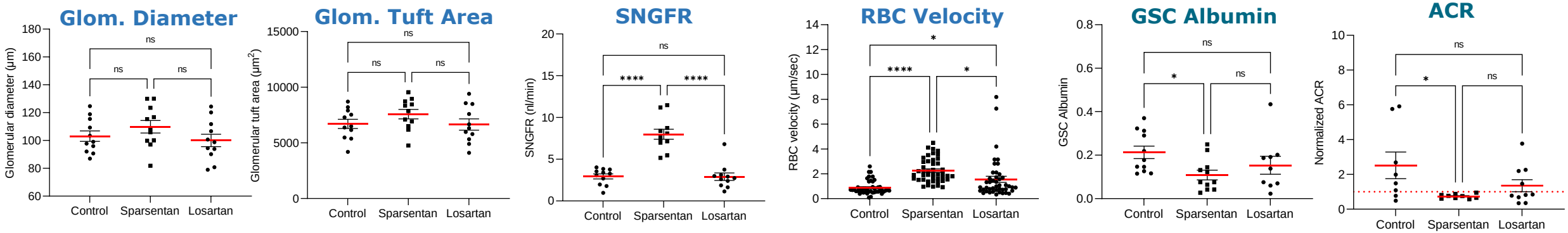
-MPM imaging
-Urinalysis

Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

FSGS-Tg mice: basal renal hemodynamic responses







Sparsentan-treatment significantly improved podocyte Ca^{2+}, AA/EA diameter, GSC, SNGFR, and ACR vs. control mice, while losartan improved only podocyte Ca^{2+} and blood flow. Glomerular diameter and tuft area trended upward with sparsentan-treatment but were not significant ($p<0.05$).

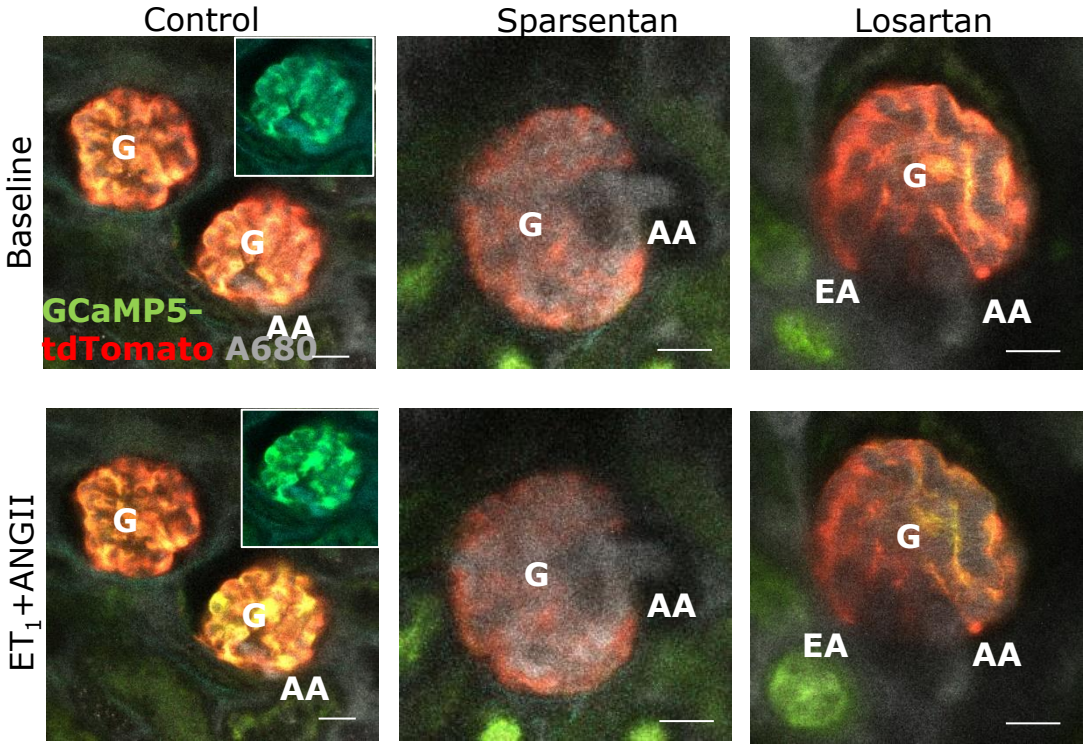
Results

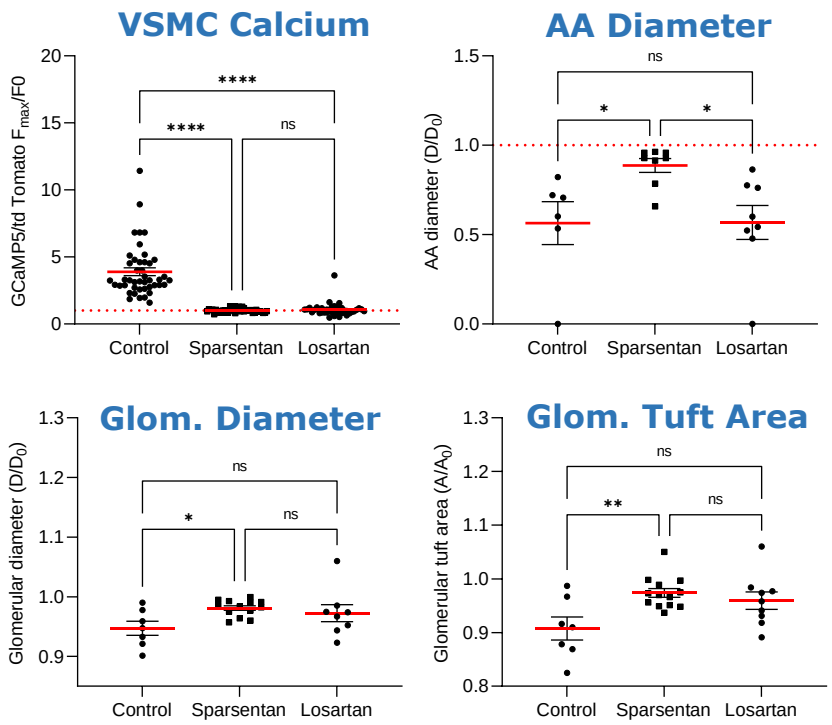
Treatment for 6 weeks po. **MPM imaging**

Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

Acute vaso-constriction: ET-1 with Ang II ia.

FSGS-Tg mice: Hemodynamic responses to acute agonist-induced vasoconstriction





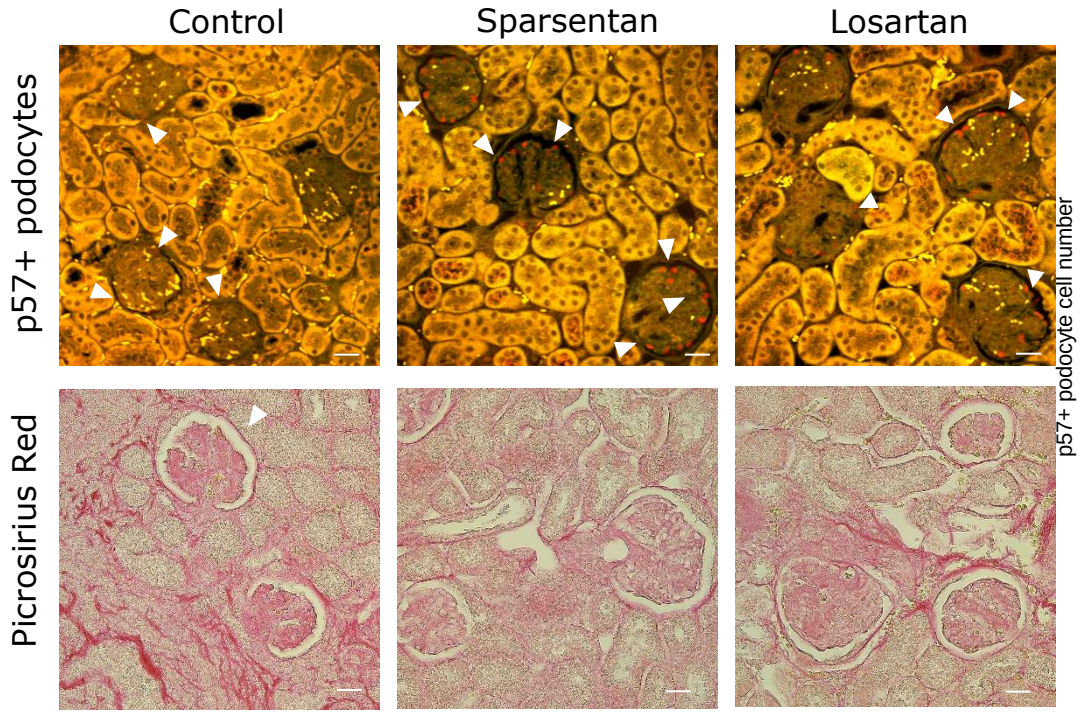
Sparsentan blocked agonist-induced AA vasoconstriction, increased Ca^{2+} and decrease in glomerular diameter and tuft area. Losartan inhibited Ca^{2+} only.

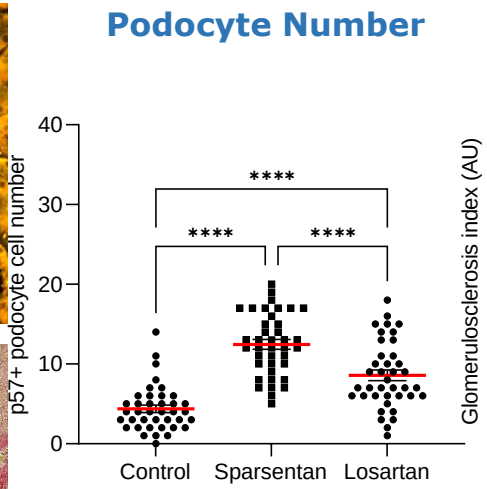
Results

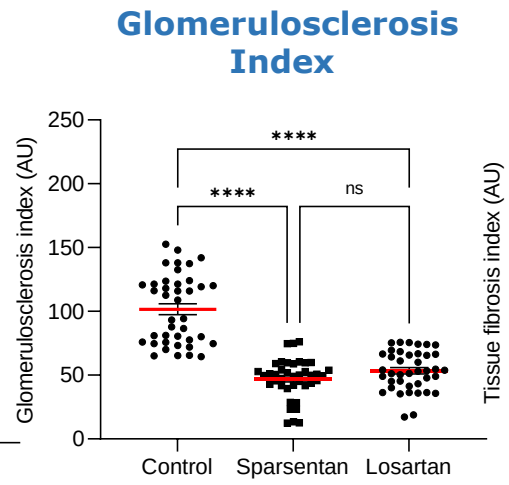
Treatment for 6 weeks po. **Histology**

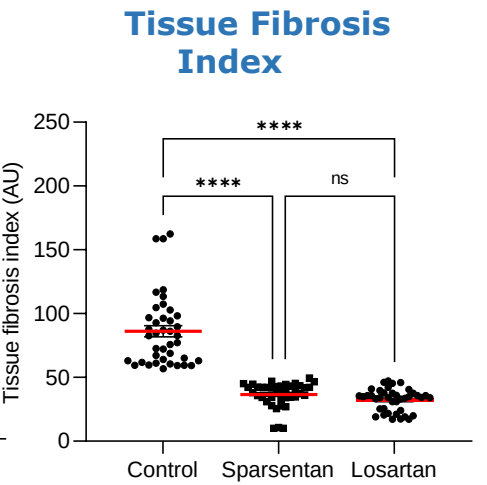
Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

FSGS-Tg mice: podocyte number, glomerulosclerosis, and tissue fibrosis









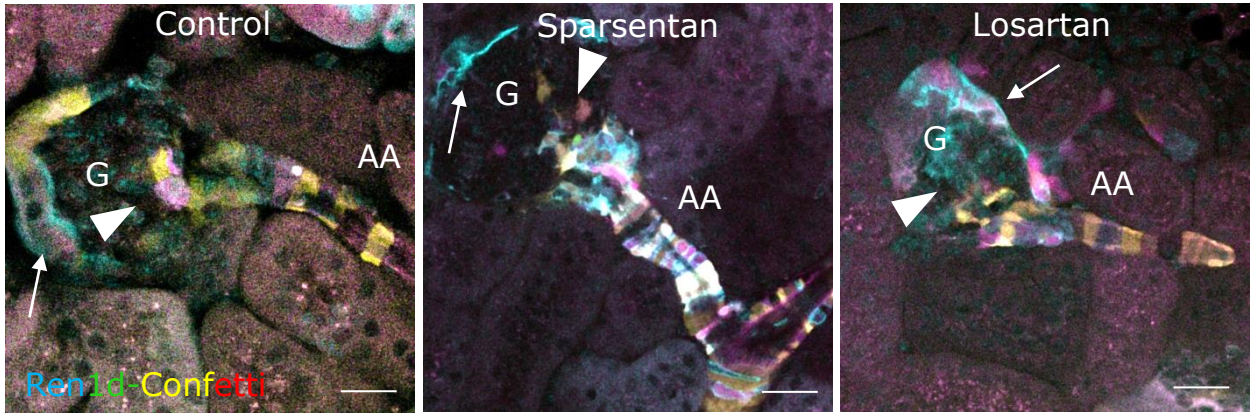
Sparsentan-treatment significantly ($p<0.05$) improved podocyte number, GS and tissue fibrosis index vs. control, and more than losartan.

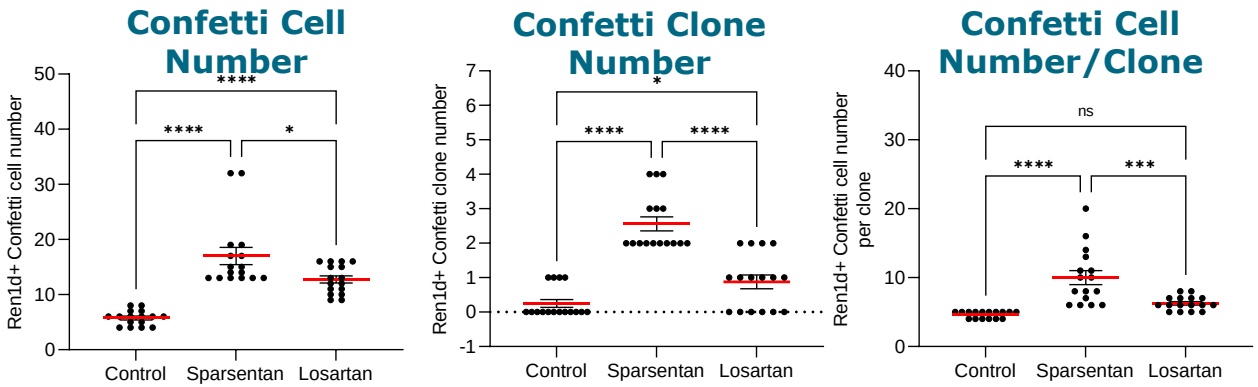
Results

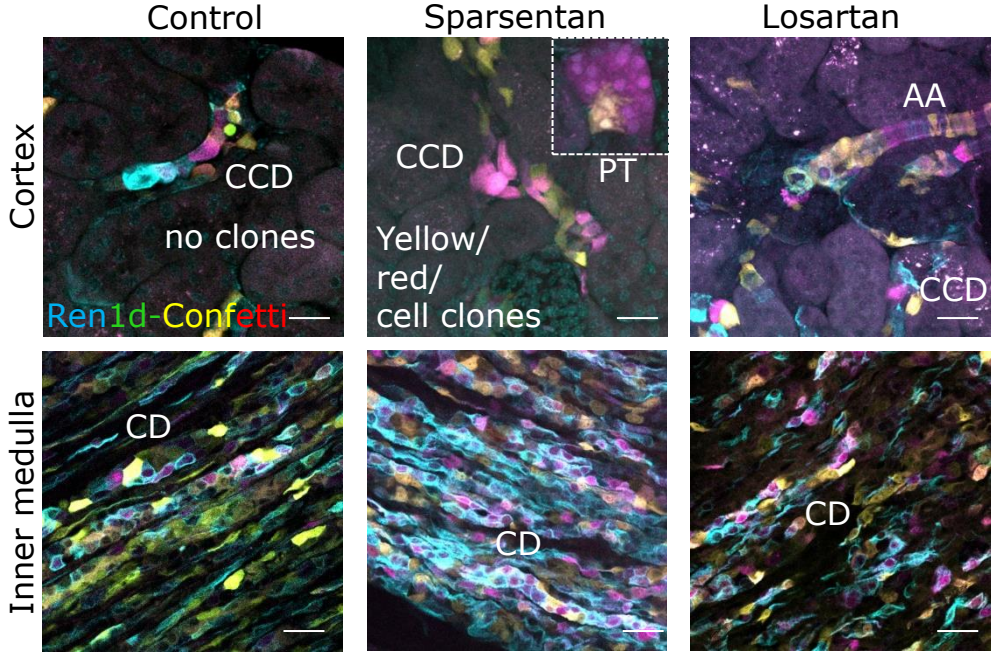
Treatment for 2 weeks po. **MPM imaging**

Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

Healthy Ren1d-Confetti Tg mice: physiological tissue remodeling responses







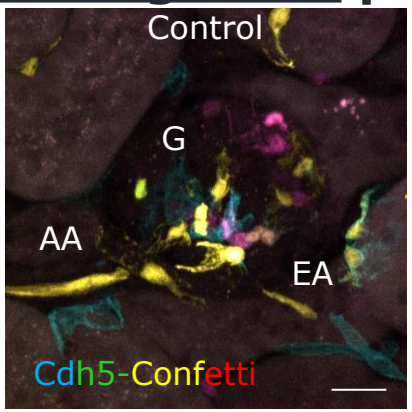
Sparsentan-treatment significantly ($p<0.05$) enhanced clonal vascular/glomerular/tubular remodeling vs. control, and more than losartan.

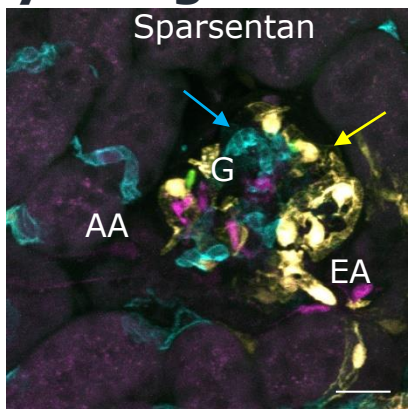
Results

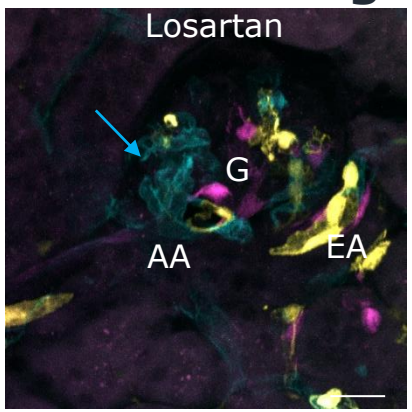
Treatment for 2 weeks po. **MPM imaging**

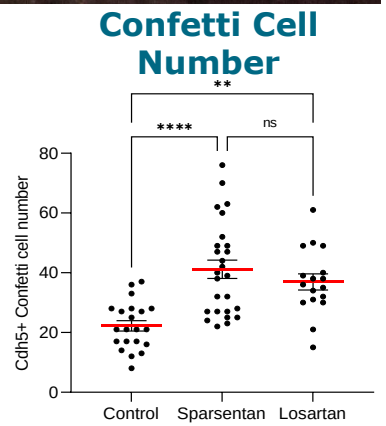
Vehicle Ctrl / Sparsentan / Losartan
(120 mg/kg/day) (10 mg/kg/day)

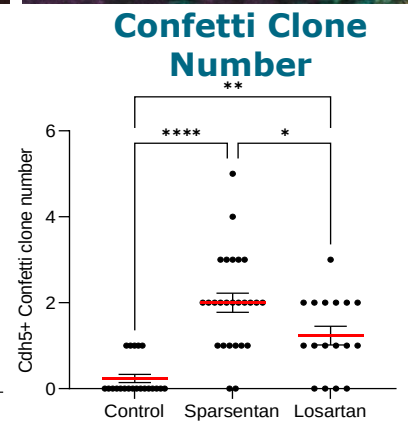
Healthy Cdh5-Confetti Tg mice: physiological tissue remodeling responses

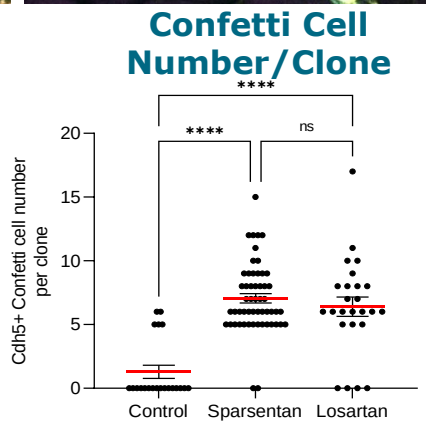












Sparsentan-treatment significantly ($p<0.05$) enhanced clonal glomerular endothelial remodeling vs. control, and more than losartan.

Summary and Conclusions

- MPM imaging directly visualized several mechanisms underlying beneficial antiproteinuric and structural effects of sparsentan in both FSGS-Tg and in the normal (Healthy-Tg and Ren1d/Cdh5-Confetti-Tg) mouse kidneys.

- Sparsentan-treatment had a greater impact on reduction in proteinuria (ACR) and increase in podocyte protection in the FSGS-Tg model than losartan. This glomerular hemodynamic pattern was driven by both AA and EA dilation resulting in an increase in capillary blood flow and SNGFR.

- The Healthy-Tg models suggest mechanisms involving antagonism of ET-1 in addition to Ang II and activation of resident progenitor cells and tissue remodeling for sparsentan being more effective in attenuating podocyte injury and renal disease. These nonclinical findings suggest multiple layers of renal protective actions by dual ET_AR and AT_1R antagonism.

Key to Abbreviations

Significant = $p<0.05$
* $p<0.05$
** $p<0.01$
*** $p<0.005$
**** $p<0.0001$

- AA = Afferent arteriole
- Alb GSC = Albumin glomerular sieving coefficient
- CCD = Cortical collecting duct
- EA =Efferent arteriole
- PT = Proximal tubule
- SNGFR = Single Nephron GFR
- VSMC = Vascular Smooth Muscle Cell